Ubiquity Lab

Introduction and Background:

The idea of ubiquity is one that has been with Microbiologists since the time of Louis Pasteur and his many colleagues before and after him where they tried to prove the existence of life that is smaller that what the naked eye could see. Ubiquity is a term that stems from the Latin word “ubique” that means to be present everywhere. This term describes what Pasteur and other microbiologist believed and that was how microorganisms where found everywhere and can survive on almost every part of our planet. These microorganisms can be found in various shapes and sizes, and prokaryotic or eukaryotic.

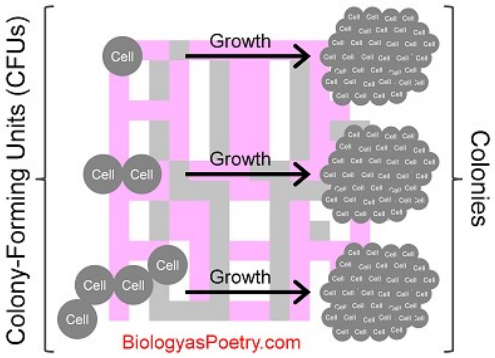
In today’s experiment, you will be asked to identify and differentiate between three different types of microorganisms: bacteria, mold and yeast. You will be using a nutrient *agar* to culture and observed these three types of microorganisms and how they can be found on a variety of different surfaces.

Agar Plates

Agar is a complex polysaccharide that is derived from marine alga and will not be degraded by the microorganisms. This agar can be manipulated by adding additional nutrients like glucose, amino acids and other nutrients that the microorganisms will be able to metabolize and grow exponentially to the point that you will be able to see their existence with the naked eye, a microbial *colony*.

Colony Forming Units

Colonies originate as a single cell and over time will start to divide and reproduce asexually. Once you can physically see the colony with the naked eye it is then said to be a “colony-forming unit” or CFU. Below is a diagram expressing how one cell will grow and divide to from the CFUs.



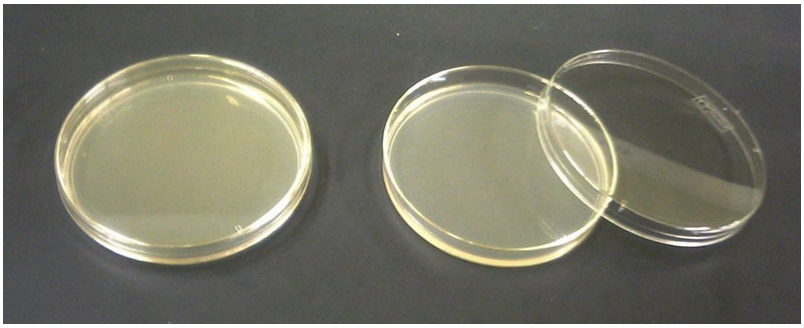
It is important to note that the colony grows in microbial numbers but the microorganism itself does not grow in size.

Countable Range on an Agar Plate

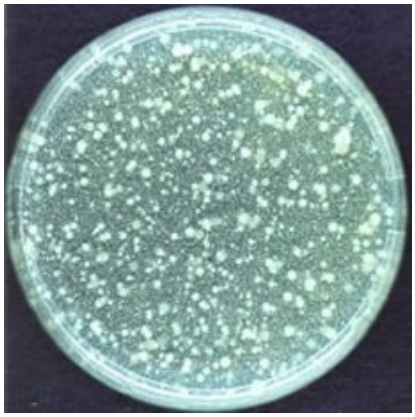
A microbiologist must accurately count the number of colonies of an agar plate and know the limitations of the plates. There will be instances where the plate may not have a single colony present or too many colonies present. The question is then what number do you record?

According to the US Food and Drug Administration *Bacterial Analytical Manual* (BAM) the lower limit can be <1 cfu, not 0 (depends on the dilution) when the microbiologist does not see a colony present.

The upper limit is also in question when you have too many microbes present. FDA's BAM recommends that when counting colonies that you can only count up to 250 CFUs and assigned the value >250 CFUs for that plate or particular organism. Any number above 250 will have skewed results due to human error and the fact that these microbes on the plate will start to compete for nutrients and space to grow. You will learn later in the semester that microbes can create toxins that may inhibit or even kill other microbes so they can have an advantage to survive on this surface possibly.



Picture 1: Represents an agar plate with no microbial growth. The total number of CFUs present is <1 CFUs.

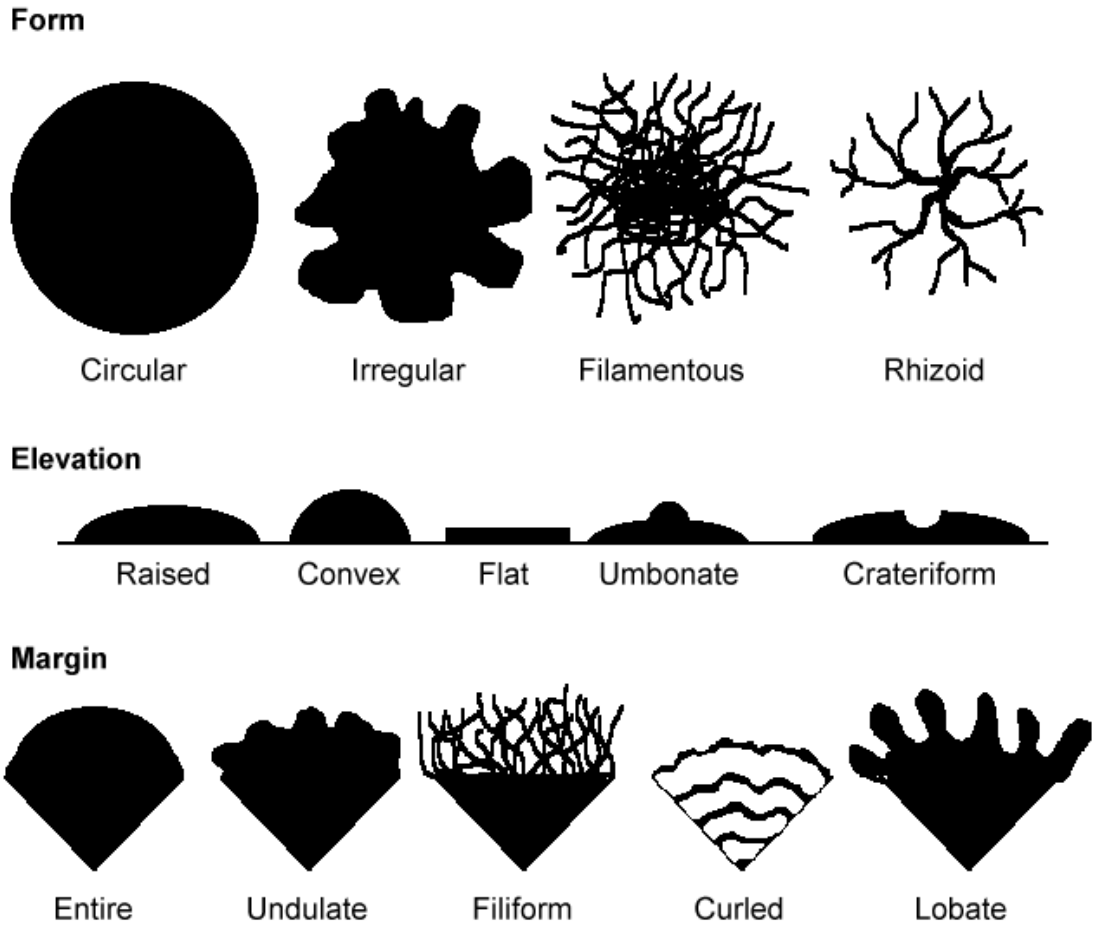


Pic 2: Represents an over grown agar plate. The total number of CFUs is >250 CFUs.

Interpreting colonies on an agar plate

There are certain characteristics that a microbiologist looks for when looking at an agar plate with colonies present. The microbiologist will look for the following elements in order to identify the colony:

- Form - What is the basic shape of the colony? For example, circular, filamentous, etc.
- Elevation - What is the cross sectional shape of the colony? You can accomplish this by turning the Petri dish on its end.
- Margin - What is the magnified shape of the edge of the colony?
- Surface - How does the surface of the colony appear? For example, smooth, mucoidal, glistening, rough, dull, wrinkled.
- Opacity - For instance, transparent, cloudy, translucent (looks like frosted glass), iridescent (changing colors in reflected light).
- Chromogenesis (the ability to create a pigment) - For example, white, black, red, green, purple, etc.



Bacteria

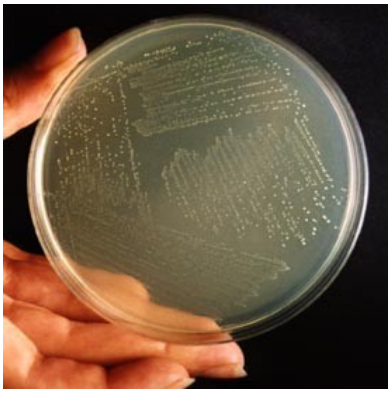
Bacteria are characterized as prokaryotic cells, which lack membrane bounded organelles and lack a membranous nucleus. They typically have a presence of a cell wall that has a carbohydrate and protein complex referred as a peptidoglycan. The type of cell wall that the bacteria have will determine what type of bacteria it is. There are 3 types of bacteria: Gram Positive, Gram Negative and Acid Fast. The three types of bacteria and how we differentiate between them will be discussed in greater detail in the future. You will be asked today to identify bacterial colonies on an agar plate.

Most bacteria on an agar plate will appear cream, white or yellow in color and will also be circular in shape. Below are a few examples of bacteria on an agar plate.

Pic 3: *Bacillus subtilis*

Pic 4: *Proteus vulgaris*

Pic 5: *Staphylococcus aureus*



Pic 6: *Streptococcus pyogenes*

Yeast

Yeast colonies are very similar characteristics to bacterial colonies except that they are eukaryotic organisms. The have a round appearance and will primarily have a white color. Yeast may also appear to have football like shapes to colony formation or an umbonate.


Picture 7: Round yeast colonies

If you cannot identify a colony as either bacteria or yeast you will need to pick that colony, stain it and then look at it underneath a microscope. If you observe a nucleus or any other membrane-bounded organelle then you have yeast. But if the microbe lacks a visible nucleus then you have bacteria instead.

Mold

Mold is a type of fungi and is also a eukaryotic organism. Their appearance can vary is size and shape and any color. They can display multiple colors as well.


Picture 8: Mold on agar plates

Pre-lab questions:

1. Why must you assign a <1 cfu if you do not see a single colony on your agar plate? In other words, why can it not be 0 cfu's?

2. Define in your own words what a CFU is.

3. What are the differences between eukaryotic and prokaryotic organisms? List as many as possible.

4. Why is agar used to identify colonies on a plate?

In today's experiment you will try and verify if indeed microbes are ubiquitous to ALL of our environments. You will be asked to formulate 4 different hypotheses for 4 different questions before the laboratory begins.

Optional: Please show your hypotheses to your instructor before class begins.

The questions are:

1. Are there microbes in the air and if so, which ones?

Hypothesis:

2. Are there microbes on every surface?

Hypothesis:

3. Can microbes be found on and in a human?

Hypothesis:

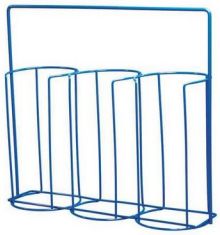
4. Is the “5 second rule” legitimate?

Hypothesis:

Materials Needed for the Ubiquity Lab

- 6 Wax Pencils

- 1 Metal Petri Dish Racks



- 2 Test Tube Racks

- 6 Metal Inoculating Loops

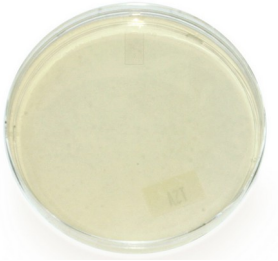
- 6 Metal Inoculating Needles

- 2 Bunsen Burners and Hoses

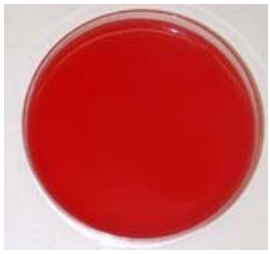
- 10 Sterile Cotton Swabs (10 per Table)

- 10 Sterile tryptone broth test tubes

- 10 Sterile tryptone agar plates



- 3 Sterile Blood agar plates



- 1 “Waste” 500ml Beakers

Procedure:

Experiment 1 - Are there microbes in the air and if so, which ones?

Day #1

1. Your instructor will conduct positive and negative controls for you at the beginning of the laboratory.
2. Each group will need to obtain 5 Tryptic Soy Agar (TSA) plates. Label the agar side of each plate with a wax pencil with the following information:

    a. Each plate needs your initials
    b. Each plate needs the date
    c. Each plate needs the class section
        i. 1 plate should be labeled 1 minute
        ii. 1 plate should be labeled 2 minutes
        iii. 1 plate should be labeled 5 minutes
        iv. 1 plate should be labeled 10 minutes
        v. 1 plate should be labeled 15 minutes.
3. Have an alarm/timer ready and set an alarm for 1, 2, 5, 10 and 15 minutes.
4. Place your plates in the middle of your bench next to each other after they are labeled. Remove the lids and start the timer.
5. Once the time is reached, please place the lid back on its respected plate.
6. Place the plates in the metal dish rack and make sure that they are placed with the **AGAR SIDE UP**.
7. Take the temperature of your incubator and record it in your lab notebook.
8. Place the metal dish rack with the agar plates in the incubator that is set at 35 degrees Celsius and let them incubate for 24 hours.

Day #2:

1. Take a colony counter provided by your instructor to your bench
2. Take the temperature of you incubator and record it in your lab notebook.
3. Remove your plates from the incubator.
4. Use the colony counter to count how many CFUs of bacteria, yeast, and mold are present and record your data. Your instructor may ask you to make a graph of your results or they

may ask to share this data with the rest of the class to find the average CFUs for the entire class.

Optional Section

a. Ignite a Bunsen burner and sterilize a metallic loop using aseptic techniques.
b. If possible, pick an isolated colony that is either bacteria or yeast and place it in a Tryptic Soy broth (TSB) test tube with a sterile metallic loop and then place it in your test tube rack.
c. Take your test tube rack to the incubator. Record the temperature of the incubator and incubate the TSB for 24 hours.

5. The used agar plates must be disposed in the BIOHARZARDOUS WASTE that is located in the back of the room. Please ask your instructor if you have any questions where to dispose this waste. It must NOT be discarded in the regular garbage.

Optional Day #3

1. Remove the test tubes from the incubator. Record the temperature of the incubator and record it in your lab notebook.
2. With a wax pencil write your initials at one end of the slide and on the **bottom (other side)** of the slide draw a circle in the center.
3. Use your metallic loop and with aseptic techniques transfer a small amount of liquid culture from the broth into the center of the circle you just drew on the slide. Spread out the drop of bacteria into a thin film
4. Flame your loop/needle immediately after you are done using it.
5. Allow the smear to air dry **completely** (do not try to rush this by using a Bunsen burner this will only damage your samples)
6. Once the slide is dry: quickly pass the slide through the flame two to three times. Do this in a quick constant motion. Do not leave the slide in the flame too long as this can damage your sample. Your sample is now heat fixed. This will kill the bacteria and also affix them to the slide.
7. Take a staining tray and bring it to your bench.
8. Flood your slide with Methylene Blue for 1 minute.
9. Gently wash off the Methylene Blue with water. DO NOT apply too much pressure. You want the microbes to stay on the glass slide, not to be washed away.
10. Take your slide and gently blot the water drops off using bibulous paper or brown paper towels. Make sure you are

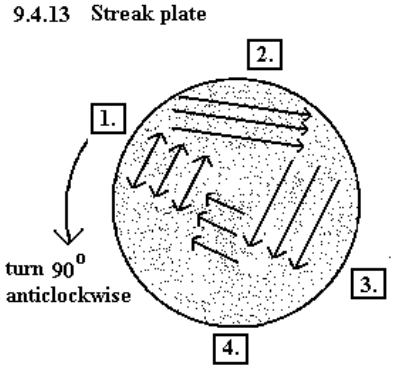
gentle with the slide so that you will not rub the microbes of the slide and not to break the glass.

11. Take the slide and identify if the microbe is a Eukaryotic or Prokaryotic organism by observing it underneath the microscope.
12. Record what you see. Draw what the microbe looks under 40X and 100X power in your lab notebook. Include the following:
    a. Color
    b. Shape
    c. Size
13. When you are finished discard the glass slide in the “Glass Waste” Beaker on the instructors bench.
14. Take any left over test tubes and place them on the instructors bench so that the instructor can properly dispose of them.

Experiment #2 - Are there microbes on every surface?

Day #1:

1. Each group will need to obtain 6 Tryptic Soy Agar (TSA) plates.
2. Each member of the group needs to identify 1 unique environment that they will want to swab.
3. Label the agar side of each plate with a wax pencil with the following information:
    a. Each plate needs your initials
    b. Each plate needs the date
    c. Each plate needs the class section
    d. Each plate should be labeled with the environment that is intended to be swabbed.
4. Now that everyone has identified a surface to test for the presence of microbes you will need a sterile swab.
5. Take the sterile swab, open it and introduce to a sterile solution broth like Tryptic Soy broth (TSB).
6. With the wet, sterile swab please go to the specified surface and swab it in a up and down, left and right to ensure that the surface is being tested properly.
7. Take the swab and inoculate the TSA plate in the following motion:



Make sure to inoculate the entire surface of the agar without stabbing it. Remember, it is a semi-solid substance that can be cut if you apply too much pressure. Be gentle.

8. Place the used swab in your “waste” beaker.
9. Replace the lid of the plate to your agar plate.
10. Take the closed agar plate and place it in the metal dish rack and make sure that the plates are place ***AGAR SIDE UP***.
11. Take the temperature of your incubator and record it in your lab notebook.
12. Place the metal dish rack with the agar plates in the incubator that is set at 35 degrees Celsius and let them incubate for 24 hours.

13. Please give your instructor any used test tube broths so that they can be discarded appropriately.

Day #2:

1. Take a colony counter provided by your instructor to your bench
2. Take the temperature of you incubator and record it in your lab notebook.
3. Remove your plates from the incubator.
4. Use the colony counter to count how many CFUs of bacteria, yeast, and mold are present and record your data. Your instructor may ask you to share this data with the rest of the class to find the average CFUs for the entire class.

Optional Section

   a. Ignite a Bunsen burner and sterilize a metallic loop using aseptic techniques.
   b. If possible, pick an isolated colony that is either bacteria or yeast and place it in a Tryptic Soy broth (TSB) test tube with a sterile metallic loop and then place it in your test tube rack.
   c. Take your test tube rack to the incubator. Record the temperature of the incubator and incubate the TSB for 24 hours. Follow the instruction on page 11.

5. The used agar plates must be disposed in the BIOHARZARDOUS WASTE that is located in the back of the room. Please ask your instructor if you have any questions where to dispose this waste. It must NOT be discarded in the regular garbage.

Experiment 3 - Can microbes be found on and in a human?

Day #1:

1. Each member of the group will need to obtain 1 Blood Agar plates.
2. Label the agar side of each plate with a wax pencil with the following information:
    a. Each plate needs your initials
    b. Each plate needs the date
    c. Each plate needs the class section
3. Take a sterile swab and swab the back of your throat. Inoculate and spread the Blood Agar plate with the swab as described earlier in experiment #2. If you cannot do that, please cough repeatedly into the open Blood Agar plate.
4. Take the closed agar plate and place it in the metal dish rack and make sure that the plates are place ***AGAR SIDE UP***.
5. Take the temperature of your incubator and record it in your lab notebook.
6. Place the metal dish rack with the agar plates in the incubator that is set at 35 degrees Celsius and let them incubate for 24 hours.

Day #2:

1. Take a colony counter provided by your instructor to your bench
2. Take the temperature of you incubator and record it in your lab notebook.
3. Remove your plates from the incubator.
4. Use the colony counter to count how many CFUs of bacteria, yeast, and mold are present and record your data. In addition, please try and identify colonies that are displaying hemolysis and count how many CFUs you have. Hemolysis is derived from the Greek words ΑΙΜΑ that means blood and ΛΥΣΙΣ that means to set free. When this phenomenon occurs on the Blood Agar plate the agar will change colors from red to transparent. This is displayed in the picture below.

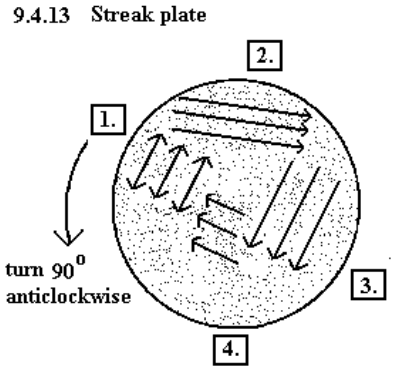
Picture #9: CFUs displaying Hemolysis.

5. The used agar plates must be disposed in the BIOHARZARDOUS WASTE that is located in the back of the room. Please ask your instructor if you have any questions where to dispose this waste. It must NOT be discarded in the regular garbage.

Experiment 4 - Is the "5 second rule" legitimate?

Day #1:

1. Each group will need to obtain 2 Tryptic Soy Agar (TSA) plates.
2. Label the agar side of each plate with a wax pencil with the following information:
    a. Each plate needs your initials
    b. Each plate needs the date
    c. Each plate needs the class section
        i. One plate should be labeled with "5 second rule Dry".
        ii. One plate should be labeled with "5 second rule Wet".
3. Take the sterile swab, open it and introduce to a sterile solution broth like Tryptic Soy broth (TSB).
4. With the wet, sterile swab please drop the swab on the floor and count to five..
5. Pick up the swab from the surface like you would pick up food. Take the swab and inoculate the TSA plate in the following motion:



Make sure to inoculate the entire surface of the agar without stabbing it. Remember, it is a semi-solid substance that can be cut if you apply too much pressure. Be gentle.

6. Place the used swab in your "waste" beaker.
7. Replace the lid of the plate to your agar plate.
8. Repeat steps 3-7 but use a DRY sterile swab instead of a wet sterile swab.
9. Take the closed agar plate and place it in the metal dish rack and make sure that the plates are place ***AGAR SIDE UP***.
10. Take the temperature of your incubator and record it in your lab notebook.

11. Place the metal dish rack with the agar plates in the incubator that is set at 35 degrees Celsius and let them incubate for 24 hours.
12. Please give your instructor any used test tube broths so that they can be discarded appropriately.

Day #2:

6. Take a colony counter provided by your instructor to your bench
7. Take the temperature of you incubator and record it in your lab notebook.
8. Remove your plates from the incubator.
9. Use the colony counter to count how many CFUs of bacteria, yeast, and mold are present and record your data. Your instructor may ask you to share this data with the rest of the class to find the average CFUs for the entire class.

Optional Section

a. Ignite a Bunsen burner and sterilize a metallic loop using aseptic techniques.
b. If possible, pick an isolated colony that is either bacteria or yeast and place it in a Tryptic Soy broth (TSB) test tube with a sterile metallic loop and then place it in your test tube rack.
c. Take your test tube rack to the incubator. Record the temperature of the incubator and incubate the TSB for 24 hours. Follow the instruction on page 11.

10. The used agar plates must be disposed in the BIOHARZARDOUS WASTE that is located in the back of the room. Please ask your instructor if you have any questions where to dispose this waste. It must NOT be discarded in the regular garbage.

Data:

You must create data tables in your lab notebook for each experiment. Each data table must have a title, organized and easy to read. Your instructor may ask to see this data table. In addition, please construct graphs for each data table using Microsoft Excel.

Include the results for your controls here:

- Negative Control: _____
- Positive Control: _____

Conclusion:

Your data has now been collected and you should be able to be able to validate or invalidate your original hypotheses. Please justify your conclusions below for each experiment.

Experiment #1:

Experiment #2:

Experiment #3:

Experiment #4:

Post Lab Questions

1. Explain why you need to conduct negative and positive controls in every laboratory experiment.

2. Did you see an increase/decrease/no change in the air plates over time? What types of organisms were present? Was there more of one particular one over the other?

3. What is hemolysis? What causes it?

4. What are the differences between bacteria and yeast?

5. What are the differences between mold and yeast?